(12) UK Patent Application (19) GB (11) 2 207 245 (13) A

(43) Application published 25 Jan 1989

(21) Application No 8816751

(22) Date of filing 14 Jul 1988

(30) Priority data
(31) 8716622 (32) 15 Jul 1987 (33) GB
8722539 24 Sep 1987
8724653 21 Oct 1987

(71) Applicant
Unilever PLC

(Incorporated in United Kingdom)

Unilever House, Blackfriars, London, EC4P 4BQ

(72) Inventors
Anthony Hugh Carr
Paul Richard Darnell
Paul James Davis
Paul William Perry

(74) Agent and/or Address for Service
D J Butler
Patent Division, Unilever PLC, P O Box 68,
London, EC4

(51) INT CL⁴
G01N 21/76

(52) Domestic classification (Edition J):
G1A A4 D6 G7 KA P1 R7 S10 S8 T2

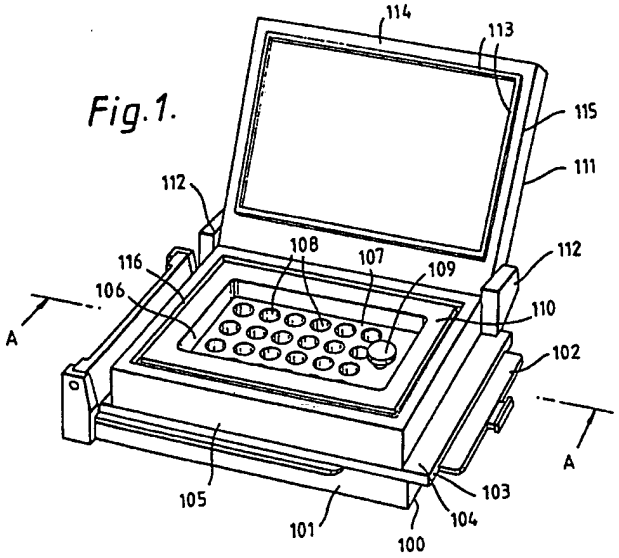
(56) Documents cited
EP A2 0207532 EP A2 0071859 US 4672039
US 4604364

(58) Field of search
G1A
Selected US specifications from IPC sub-class
G01N

(54) Specific binding chemiluminescent assay

(57) In an assay method for determining quantitatively the presence of an analyte in a sample by means of specific binding, the sample is contacted with a solid phase carrier material on which is immobilised a first binding reagent having specificity for the analyte, and with a labelled specific binding reagent which can participate in either a "sandwich" or a "competition" reaction with the first reagent in the presence of the analyte, the label participates in a chemiluminescent reaction the light output from which is used as a means for determining the extent (if any) to which the labelled specific binding reagent has become bound to the solid phase, and the light is derived from a point source and is recorded on a light-sensitive film in such a manner that the size of the image formed on the film provides a measure of the quantity of the analyte in the sample. Light recording apparatus, incorporating a multi-well micro-analytical tray and wherein the point source for each well is defined by an aperture in a perforated plate which can be situated directly beneath the tray and spaced appropriately from the film, is also described.

Fig.1.



The drawing(s) originally filed was (were) informal and the print here reproduced is taken from a later filed formal copy.

GB 2 207 245 A

Fig.1.

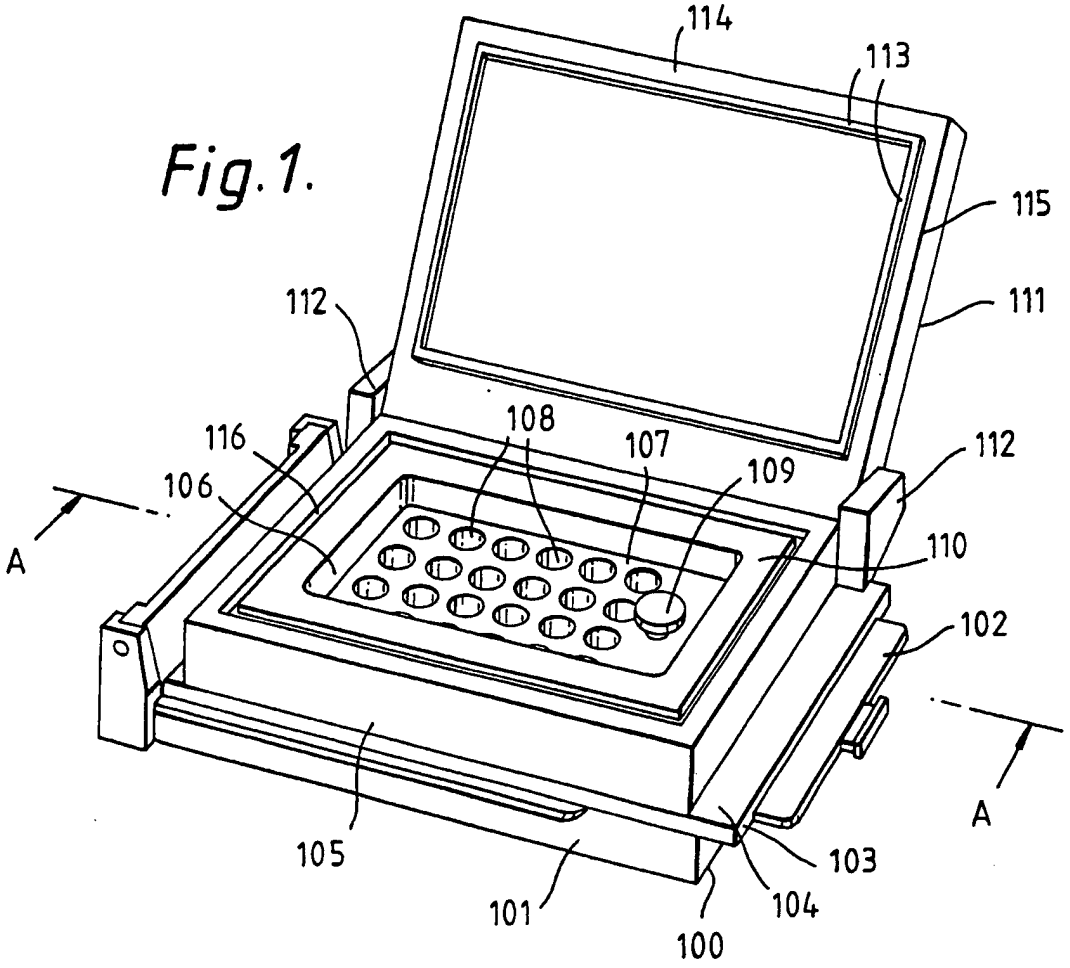
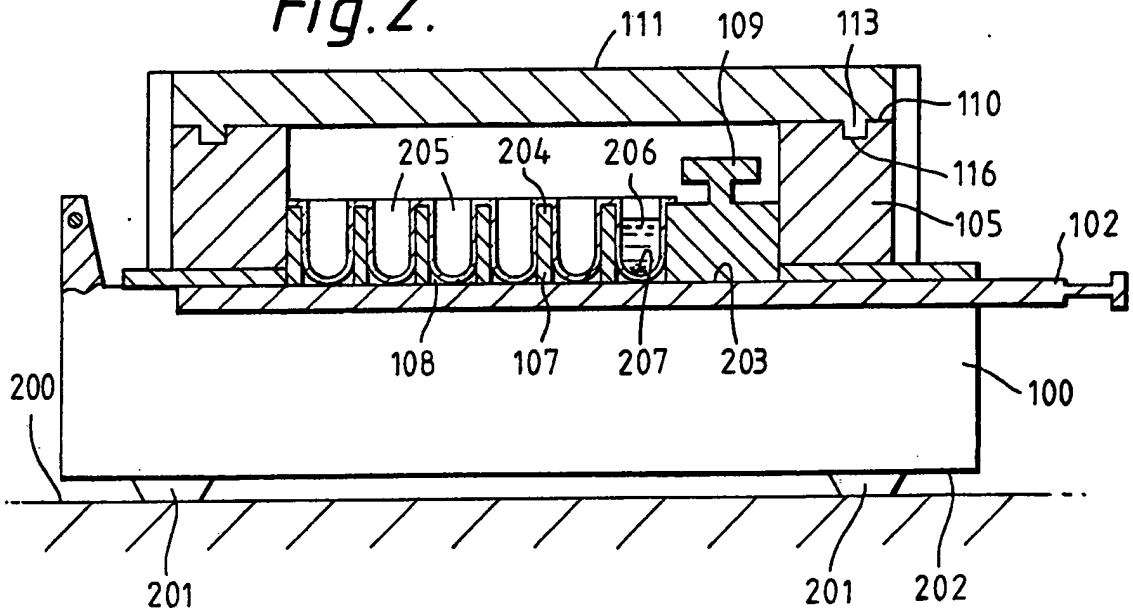

114
113
115
111
112
116
108
107
109
106
112
A
110
102
A
105
103
101
104
100

Fig.2.


111
109
113
110
205
204
206
116
105
102
108
107
207
203
200
100
201
201
202

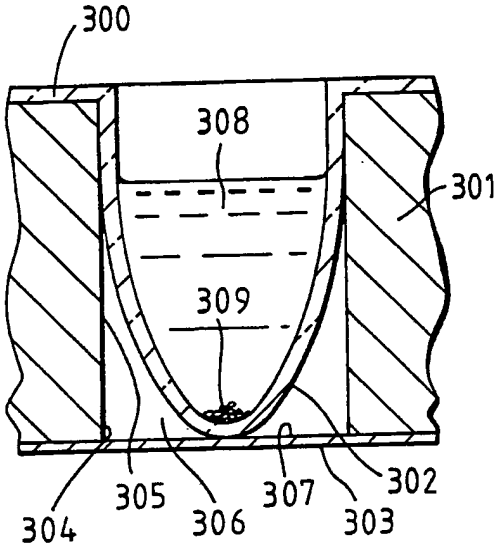


Fig.3.

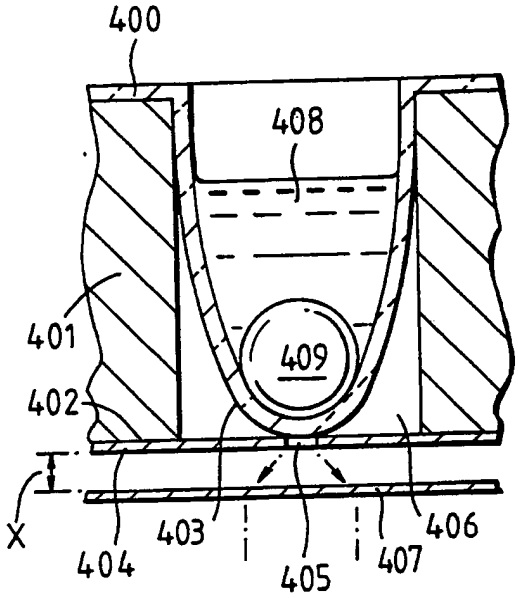


Fig.4.

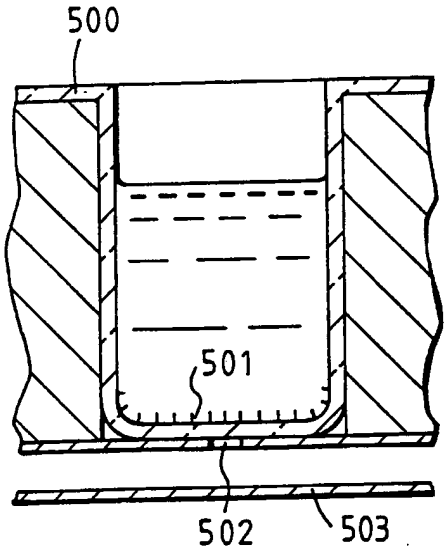


Fig.5.

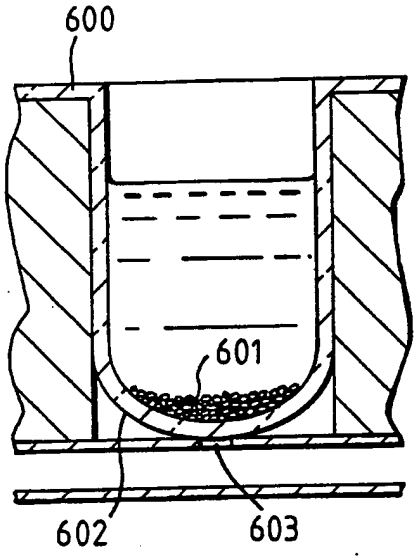


Fig.6.

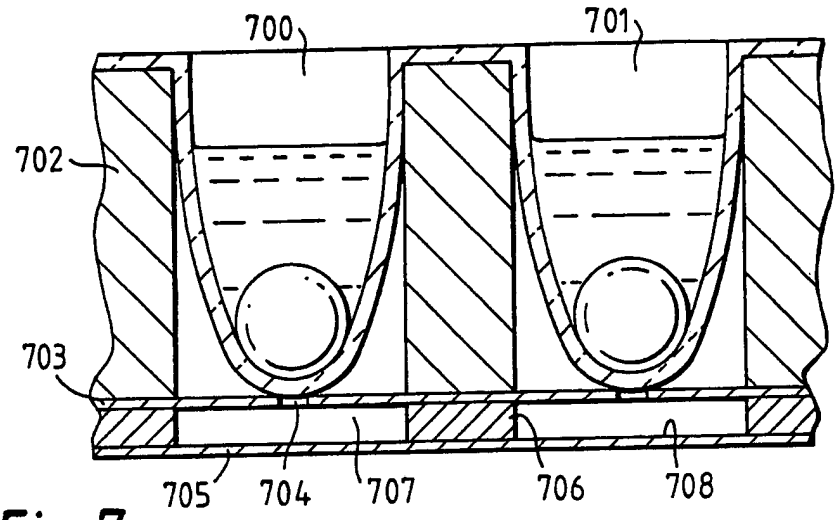

700
701
702
703
705
704
707
706
708

Fig.7.

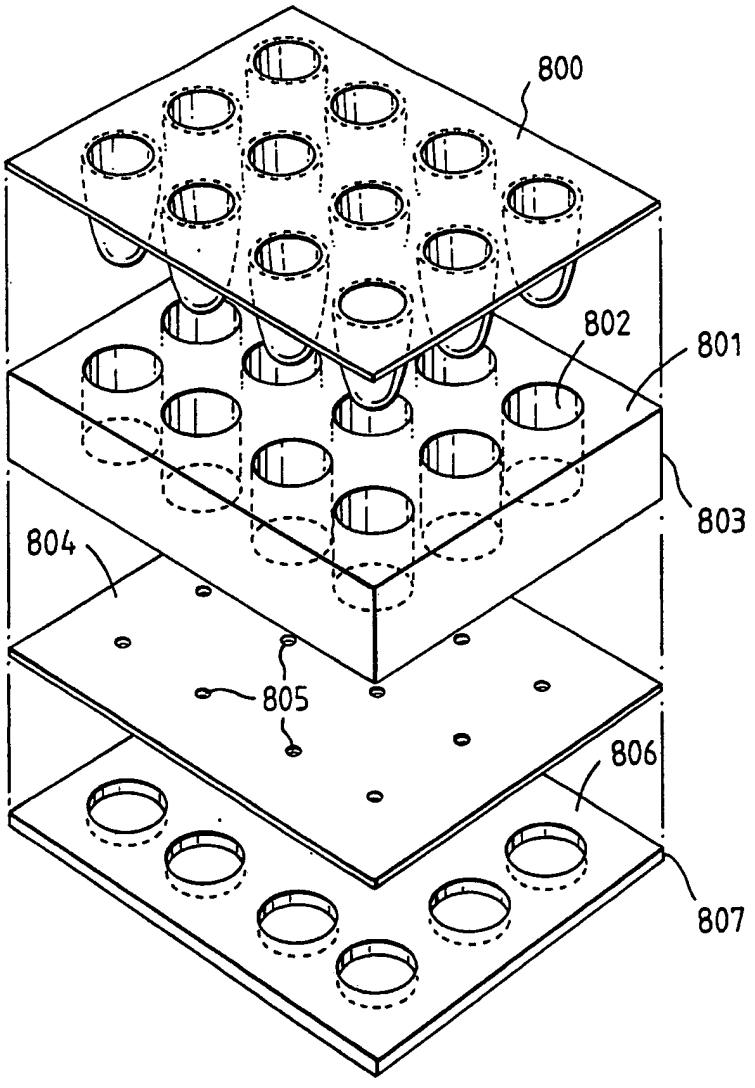

800
802
801
803
804
805
806
807

Fig.8.

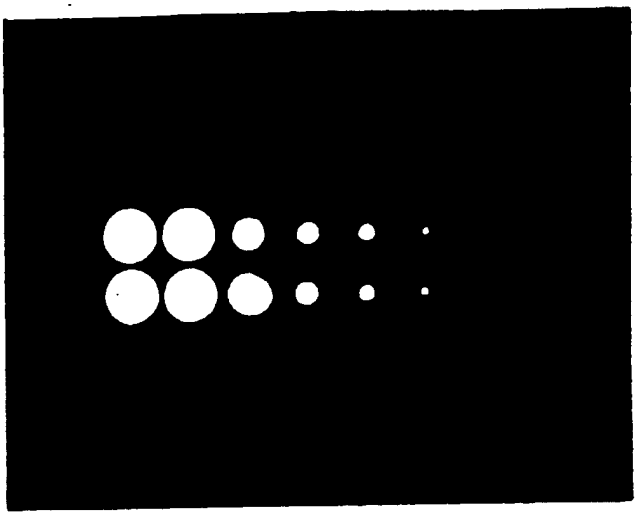


Fig.9.

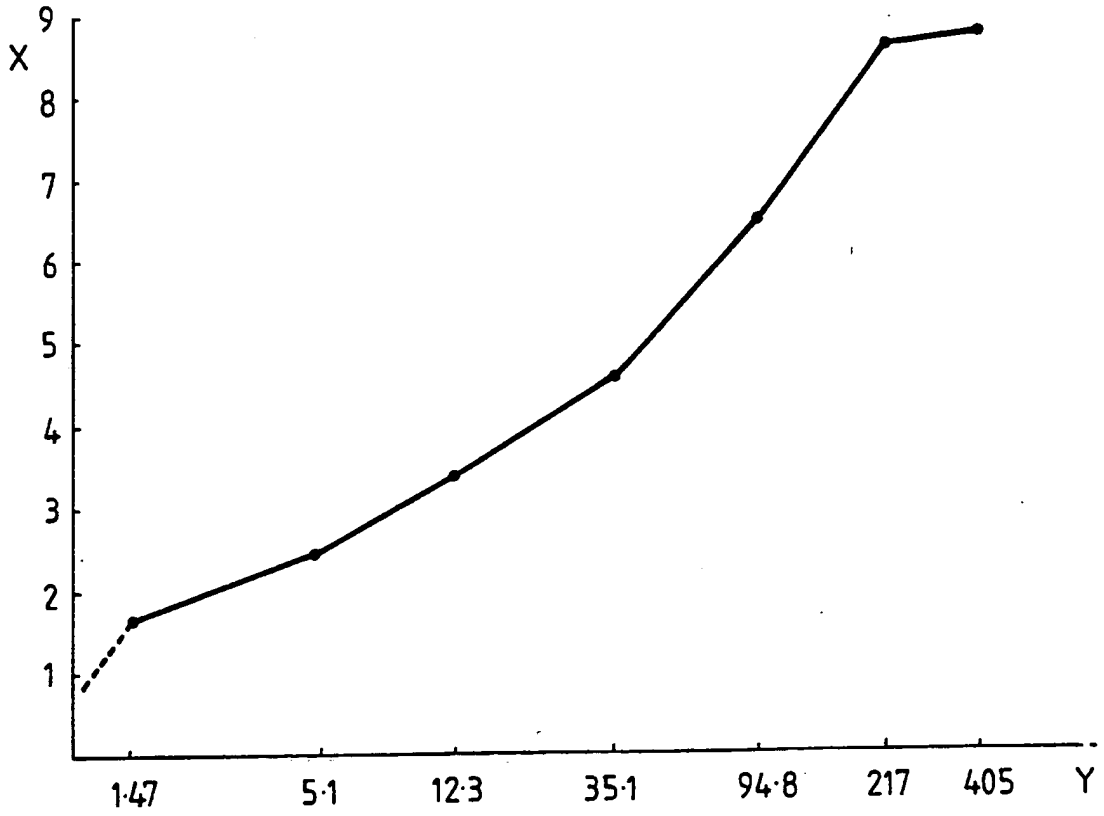


Fig.10.

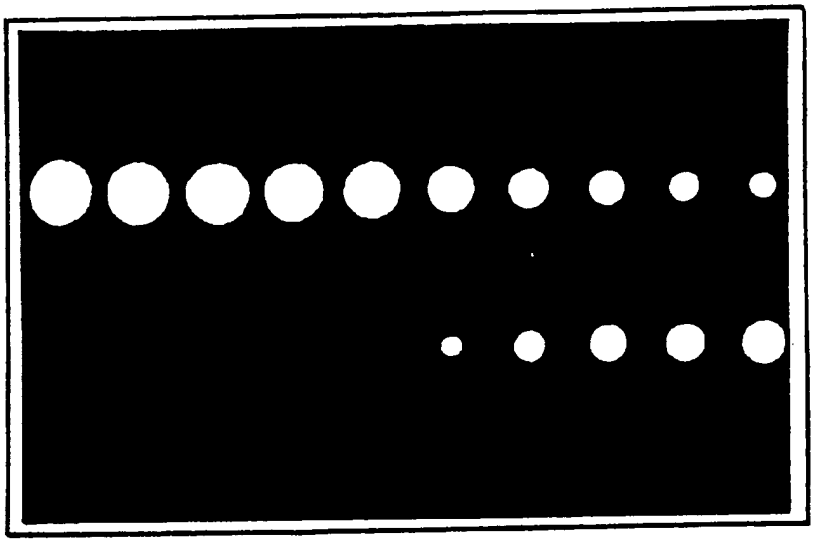


Fig.11.

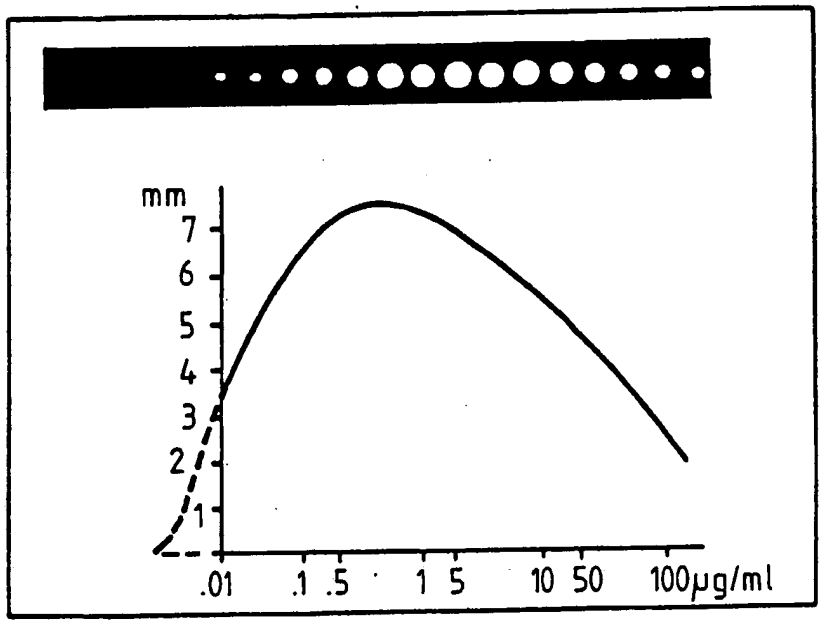


Fig.12.

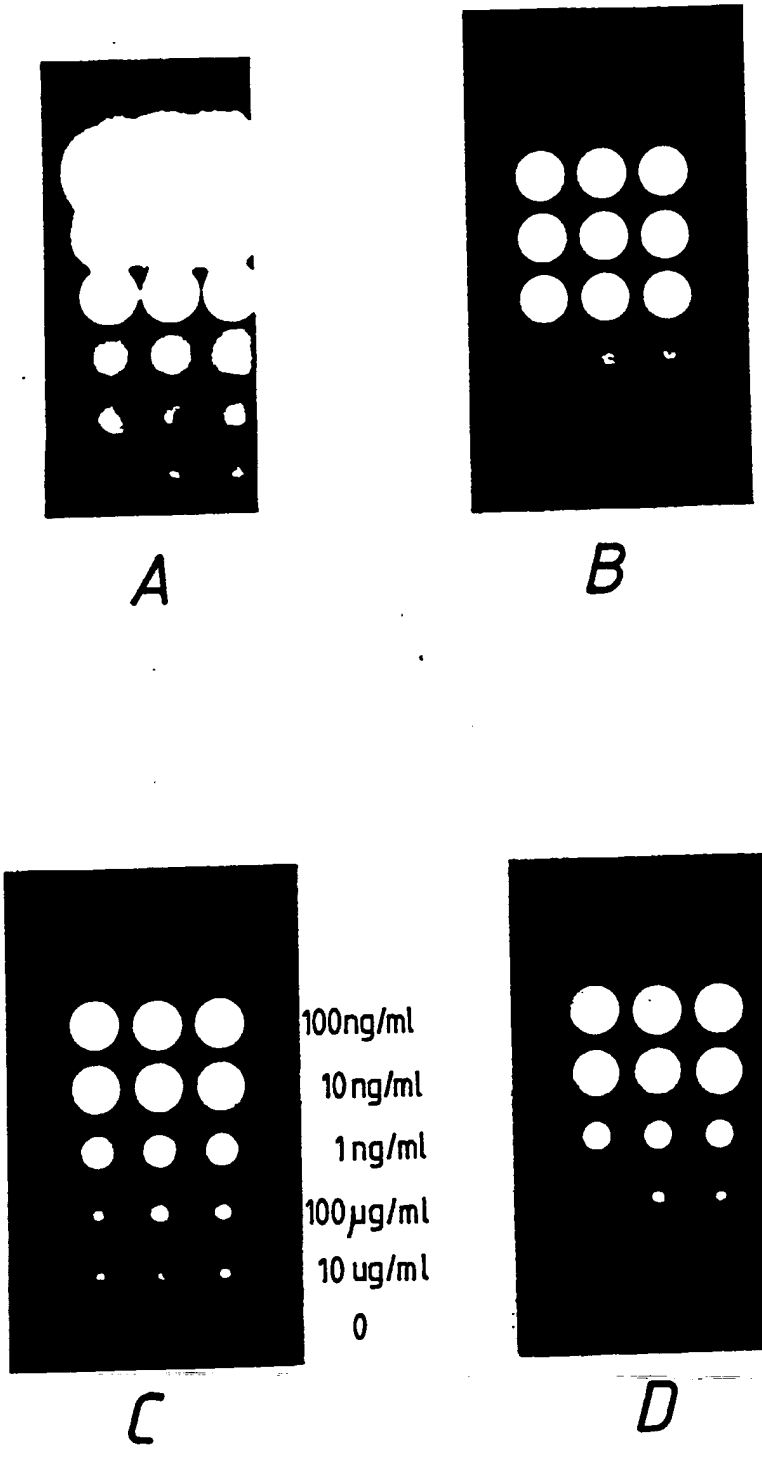


Fig.13.

ASSAYS

The present invention relates to assays involving specific binding, and particularly to immunoassays and to apparatus in which the results of such assays can be recorded.

Solid phase, e.g. particle-based, immunoassay systems are already known. These involve the qualitative and/or quantitative determination of an immunogenic species in a test sample by means of an immunological binding reaction between the immunogenic species (analyte) and one or more specific binding partners therefor, at least one of which binding partners is linked to an insoluble carrier material in contact with a liquid medium comprising the test sample. The fact that the resulting immunological complex is linked to the carrier material can be used to advantage during the subsequent determination of the extent to which the binding reaction has taken place. The determination is usually achieved by the use of a labelled specific binding partner, and the binding reaction can be

either a "competition" reaction or a "sandwich" r action, both of which are now widely used in this art.

In a typical "competition" reaction, the assay medium, which is generally aqueous, is contacted with the solid phase carrier on which is immobilised a specific binding reagent (e.g. monoclonal antibody) having specificity for the analyte to be determined, and the assay medium contains a known quantity of the analyte (or an analogue thereof possessing the identical epitopes) bearing a label and a known quantity of a sample suspected of containing the analyte. In the absence of analyte in the sample, the labelled analyte or analogue binds with the specific binding reagent immobilised on the solid phase carrier and the extent of this binding is determined from standard experiments. When the sample contains the analyte, this competes with the labelled analyte or analogue for the specific binding reagent and hence the extent to which the specific binding reagent becomes coupled to the labelled component is reduced. By comparing this with the standard situation, (i.e. the result obtainable in the absence of the analyte) a measure of the analyte concentration in the sample can be derived.

In a typical "sandwich" reaction, a first binding reagent having specificity for the analyte is immobilised on the solid phase carrier, and the assay medium, which is again generally aqueous, also contains a second binding reagent having specificity for the analyte and which bears a label. In the absence of analyte, no coupling can occur between the immobilised reagent and the labelled reagent. When the analyte is present it acts as a bridge between the immobilised and labelled specific binding reagents and results in indirect coupling of the labelled reagent to the solid phase. The extent of this coupling provides a measure of the analyte concentration in the sample. The

specificities of the immobilised reagent and the labelled reagent for the analyte can be different, but, if the analyte molecule possesses more than one identical epitopes, it is possible to use the same specific binding reagent both in the immobilised form and in the free-labelled form.

European patent specification No.0149565 (Amersham International PLC) describes assays such as immunoassays and two-site immunometric assays, performed using a labelled reagent and another reagent bound to magnetically attractable particles which are suspendable but insoluble in a liquid assay medium, in which after the labelled reagent has become partitioned between the liquid phase and the particles in proportions which depend on the concentration of an analyte in a sample, the liquid phase is removed and the particles are re-suspended in another liquid medium and the concentration of label observed. The assay method described is particularly suitable for fluorescent and chemiluminescent label systems, and can conveniently be performed in multi-well plates in which the wells are optically screened from one another, the observations being made from above or below the wells. On page 5 of EP 0149565 it is stated that the centrifuging of micro-titre plates is inconvenient and also that it is difficult to make useful measurements of any optical signal generated from the pellet of solid material which results from bringing down a particulate reagent out of suspension. For this reason, the authors of EP 0149565 maintain that the use of magnetically attractable particles which are resuspended in liquid prior to observing the signal permits the widespread use of micro-titre plates for immunoassays.

EP 0149565 is concerned with quantitative assays in which the fluorescent or chemiluminescent signal is read

numerically by means of instruments. It may well b true that such readings are more easily taken from a particulate solid phase in the dispersed state.

However, in contrast to this prior art, we have found that quantitative assay results can be obtained using a luminescent signal-generating system and a solid phase which is localised, rather than dispersed throughout an assay medium, if the signal generated by the localised solid phase is recorded using a light-sensitive film. The need for expensive optical recording equipment is therefore avoided, and the developed film can provide an easily storable permanent record of the analytical result. This is especially useful where the large-scale screening of samples in hospitals, clinics and medical laboratories is concerned. The present invention provides specific techniques whereby robust and simple assays involving a light-sensitive film as the recording means can be achieved.

Essentially, the invention provides an assay for an analyte in a sample, wherein a luminescent signal is used in the determination of the assay result and the signal is recorded on a light sensitive film, the recorded light being derived from a point source in such a manner that the size of the image formed on the film provides a measure of the quantity of an analyte in the sample.

More specifically, the invention provides An assay method for determining the presence of an analyte in a sample by means of specific binding, using a solid phase carrier material on which is immobilised a first binding reagent having specificity for the analyte, and a labelled reagent which participates in ither a "sandwich" or a "competition" reaction with the first reagent in the pres nce of the analyte, and the label participates in a

chemiluminescent reaction the light output from which is used as a means for determining the extent (if any) to which the labelled reagent has become bound to the solid phase, the light is recorded on a light-sensitive film and the light is derived from a point source in such a manner that the size of the image formed on the film provides a measure of the quantity of the analyte in the sample.

By a point source, we mean that the area of sensitised solid phase presented to the light-sensitive film is substantially smaller than the area of light sensitive film that is exposed to the light source. In practice, the area of the solid phase presented to the film is preferably not greater than about 50% of the exposed film area, and more preferably is not greater than about 25%. This is further explained by way of example below.

In one embodiment of the invention, the solid phase itself defines the point source. Preferably this is achieved by localising a particulate solid phase carrier, to which the light emitting entity has become bound, in a small region in a vessel. For example, if the assay is conducted in a small well, for example, one of an array of wells in a typical transparent multi-well tray, at the end of the assay procedure the particulate solid phase can be localised in a tightly defined region at the base of the well and the small mass of accumulated solid phase acts as the point source. This can readily be achieved in practice using wells that have a much narrower cross-section at the base than at the top. Typical examples will be wells that are sharply tapered or which terminate at the bottom in a round bottomed or conical form. The internal shape of the well therefore constrains

the small quantity of localised particulate solid phase into a tightly defined mass at the base of the well. In this embodiment, we have found that surprisingly accurate quantitative results can be obtained by placing the well or array of wells immediately adjacent to a light sensitive film which can record light emitted from the point source defined by the particulate carrier. Preferably the light sensitive film is in direct contact with the underside of the well.

In an alternative embodiment of the invention, the point source is defined by an aperture placed immediately adjacent the exterior of the vessel in which the assay is conducted. In this embodiment the solid phase carrier does not itself define the point source, and can therefore exist as a substantially larger mass of material if desired. Indeed, the carrier material can comprise a particulate solid phase in dispersed form if desired. Alternatives are a localised particulate solid phase, a larger single particle of solid phase such as a bead, or a permanently localised solid phase such as a sensitised inner wall of the vessel in which the assay is conducted. In all of these embodiments, the external aperture defines the point source from a larger region of light-emitting material. In order to achieve the necessary quantitative nature of the assay result, in this embodiment it is necessary to ensure that the light sensitive film is spaced away from the aperture rather than being placed in direct contact with it. The distance between the film and the aperture should not be so great that when any light that emerges through the aperture reaches the film, its intensity is too low to give rise to any useful image on the film. In a typical practical situation, using an array of wells in a conventional transparent multi-well plate as the vessels in which individual assays are conducted, the aperture can b provided by means of a

perforated plate placed directly beneath the wells. Each aperture should be at least approximately on the central vertical axis of its associated well. The apertured plate should be in direct contact with the underside of the wells, and the film should be located beneath the apertured plate, but spaced therefrom by the critical distance to allow a quantitative result to be obtained. In the typical practicable situation, using conventional wells having an internal diameter at the top about 8 to 10mm, the apertures should have a maximum dimension, e.g. diameter, of at least about 0.5mm. Preferably the maximum dimension is not greater than about 3mm. The apertures should more preferably have a maximum dimension of about 1 to about 2mm. The distance between the underside of the apertured plate and the light sensitive film should be at least about 0.5mm, and in general need not exceed about 5mm. In general, the optimum distance will be in the range 1 to 3mm. The skilled reader will appreciate that in any practical arrangement, simple experiments will need to be conducted before the optimum values of these parameters can be ascertained. Preferably a perforated spacer is provided between the apertured plate and the film in order to maintain accurately the necessary distance between the aperture and the film.

An advantage of this second embodiment over the first, is that the aperture defines the point source and hence the point source is independent of the actual body of solid phase generating the light. Greater flexibility of operation is thereby achieved. Moreover, if desired, the solid phase can be chosen such that it also acts as a screen to prevent extraneous light, e.g. generated by unbound label also present in the medium, from gaining access to the aperture. For example, if the solid phase is a single large opaque bead, almost filling the lower

region of the vessel, only light generated from the underside of the bead can access the aperture.

If the localised solid phase is at the bottom of a tube and the film is placed directly beneath the tube, the light will produce a spot on the film which will vary in area according to the amount of light emitted from the label that has become bound to the carrier. A plurality of assay vessels can be examined simultaneously in this way, using a single piece of film, and the relative light output from each vessel can be estimated by comparing the spots on the film.

Accordingly, in one embodiment, the invention provides an assay method wherein, after appropriate incubation with a sample and a soluble or dispersible labelled specific binding reagent, a particulate solid phase carrier material bearing an immobilised specific binding agent is localised within a liquid medium adjacent to a light-sensitive film to enable light to be detected from a point source, the label being capable of participating in a chemical reaction that results in the generation of chemiluminescent light and the liquid medium containing such additional reagents as may be necessary to cause the reaction to occur.

In another embodiment, the invention comprises a method for assaying for an analyte which method comprises the steps of:

(a) preparing an aqueous assay medium containing;

i) a particulate solid phase carrier material on which is immobilised a specific binding reagent for the analyte to be determined, the particulate solid phase carrier being

suspendable in the aqueous assay medium but readily localisable therein;

ii) a sample suspected of containing the analyte to be determined; and

iii) a labelled reagent soluble or dispersible in the assay medium which is either a second specific binding reagent capable of participating with the analyte and the first specific binding agent in a "sandwich" reaction, or is the analyte itself or an analogue of the analyte, and the label is a chemiluminescent reactant;

(b) incubating the assay medium to enable the labelled reagent to become bound to the particulate solid phase carrier;

(c) localising the particulate solid phase carrier within the assay medium;

(d) replacing or diluting the assay medium using a liquid medium, containing or together with such additional reactants as may be necessary to cause the chemiluminescent reaction to occur; and

(e) measuring on a light-sensitive film the chemiluminescent light emitted from a point source comprising label bound to the particulate solid phase carrier in the localised state.

In a modification of the above procedures, the incubation is performed in two stages, involving an initial incubation of the particulate solid phase carrier material and the sample in the absence of the labelled reagent, at least the bulk of the sample then being

removed and replaced by a liquid medium containing the soluble or dispersible labelled reagent, and a second incubation being performed, whereafter steps (c) to (e) of the above sequence are conducted. A localisation step should be performed before the bulk of the sample is removed between the two incubation steps. As a further or alternative modification, the addition of one or more, or indeed all of, the reagents necessary to cause the chemiluminescent reaction to occur can be made earlier in the procedure, e.g. during stage (a) or when the labelled reagent is being added in a two-stage incubation.

Preferably the particulate solid phase carrier comprises magnetically attractable particles.

The use of "camera" devices to record the results of luminescent reactions in analytical procedures is already known. Such devices are described, for example, in European patent application No. 0071859 and UK patent No. 2169704. The latter document describes a recording apparatus comprising a holder for a multiplicity of reaction vessels, said holder comprising a plate having an array of holes therein for receiving an array of reaction vessels, a housing for receiving the holder, said housing being adapted to prevent entry of stray light and including an openable cover, means for holding a photographic film adjacent the underside of the plate, and a removable shutter for interposition between the film and the plate, said removable shutter being moveable between a closed position and an open position in which latter position the film is exposed in use, and said plate being arranged (a) to rest on and be supported by said shutter when the latter is in its closed position and (b) to drop towards the photographic film when the shutter is moved to its opened position. Such apparatus has not previously

been used to provide quantitative analytical results by recording light emitted from point sources.

The present invention provides an improved device for determining the result of a specific binding assay, utilising the light output of a chemiluminescent reaction as a means for determining quantitatively the presence of an analyte in a sample, wherein the device comprises a holder for a multiplicity of reaction vessels, the holder comprising a first plate having an array of holes therein for receiving an array of reaction vessels, a housing for receiving the holder, the housing being adapted to prevent entry of stray light and including an openable cover, means for holding a photographic film adjacent the underside of the plate and a removable shutter for interposition between the film and the plate, said removable shutter being moveable between a closed position and an open position in which latter position the film is exposed in use, and interposed between the base of the holder and shutter is a perforated second plate the individual perforations of which each provide an aperture beneath an individual hole in the holder, each aperture having an area substantially less than that of its associated hole in the holder, together with means for maintaining a predetermined distance between the film and the perforated plate when the shutter is moved to its open position.

Preferably, the distance maintaining means comprises a perforated third plate in which the perforations are substantially larger than the apertures in the second plate, each perforation in the third plate being situated directly beneath an aperture in the second plate. Preferably, th triple combination of the plates is arranged to rest on and be supported by the shutter when the latter is in its closed position, and to drop on to

the photographic film when the shutter is moved to its opened position.

The light-sensitive film should be extremely "fast", to ensure that minute amounts of light can be recorded and differentiated to give quantitative information. Films are now commercially available having speeds of, for example, 20,000 ASA rating, and these are very suitable.

"Polaroid" film is very suitable and convenient, and a camera device as just described can be based on a standard commercially-available "Polaroid" camera back. However, the normal film print size obtained in this manner is insufficient to cover the whole area of a standard laboratory 96-well (8x12 well) multi-well plate, and it is therefore advisable to use plates having a smaller number of wells, e.g. by using standard plates that have been cut down to a reduced size.

If desired, any risk of interference by light from any unbound label remaining in the bulk of the sample can be minimised by incorporating in the assay medium an attenuator which blocks or absorbs such light.

In one embodiment, the invention provides a particle-based immunoassay method which utilises a specific binding partner which is labelled such that the label continuously generates a chemiluminescent signal in the test medium, which signal is masked or quenched or otherwise suppressed while the labelled binding partner is uniformly dispersed within the test medium, and in which method, following the binding reaction, a local concentration of the particulate carrier material is established in the test medium such that the local concentration of complexed labelled binding partner carried by the particles provid s a local light flux that

is sufficient to overcome the local signal-suppressing ability of the test medium and hence to provide a detectable chemiluminescent signal the magnitude of which is dependent on the extent of binding that has taken place between the immunogenic species under test and the specific binding partner carried by the particles. In this embodiment, an immunoassay method of the invention is therefore characterised by the features:

a. the label generates light continuously while in the test medium;

b. while the label is dispersed in the test medium, irrespective of whether the label is free or bound in a complex linked to the carrier material, the light is masked or quenched or otherwise suppressed;

c. complexing of the labelled binding partner with the other binding partner does not of itself significantly alter the light generated by the label; and

d. a detectable light signal is produced only when the carrier material, to which is linked the complexed label, is localised in a comparatively small zone within the test medium.

The solid phase, e.g. the particulate carrier, initially has access to the bulk of the test medium, and hence can be under the influence of any light-suppressing activity that the bulk medium possesses. Subsequently, the solid phase is placed in a situation wherein it has relative freedom from the bulk sample and hence is less influenced by the light-suppressing effect. The movement of the solid phase is the only action that needs to be

performed in order to give a readable result. Constant signal production by the label itself obviates the need to add further reagents or to provide further stimuli after particle localisation has taken place and provides maximum sensitivity of detection with the minimum complexity or number of operations. The simple act of physically localising the carrier particles is all that is required in order for a detectable light signal to be produced. The suppression of the light generated by the labelled binding partner when dispersed in the test medium is a most important feature of this embodiment of the invention.

The invention utilises a label which is chemiluminescent or which participates in a chemiluminescent reaction with other components of the medium. If the medium is opaque or at least insufficiently translucent for the light to be observable when the labelled specific binding partner is evenly dispersed in the medium, an optical signal will not be detectable. Alternatively, the medium can contain a chemical species that interacts with a component of the light-generating system, and quenches or inhibits the production of an optical signal. Subsequent localisation of the particulate carrier to which is linked a complex involving the labelled specific binding partner can give rise to a localised optical signal which is of sufficient intensity to be detectable.

Such a system can be provided using, for example, horseradish peroxidase as the label, with luminol and a peroxide (such as H_2O_2) in the medium. The medium can contain a dye, sufficiently chemically inert not to be affected by the peroxide reaction, which gives the medium an optical density at an appropriate wavelength that is sufficiently high (preferably O.D. of at least about 2) to

render the light generated by the dispersed label insignificant in comparison with that observable from a localised concentration of the label. The use of dyes (attenuators) which absorb light at wavelengths including that of the emitted luminescence in immunoassays involving luminescent reactions as the labelling system, is described in EP 0165072. Alternatively, the test medium can contain a chemical reagent that interferes with the generation of the luminescence.

A further way in which the chemiluminescent signal can be reduced or eliminated in the bulk test sample is to include a quencher reagent in the sample. For example, if horseradish peroxide enzyme is used as the label, reacting with peroxide and luminol in solution to produce visible light, the reaction can be inhibited by incorporating a competing reagent, such as gallic acid or thiodiglycollic acid, in the sample.

It can be advantageous to incorporate a fluorescer system that can absorb the chemiluminescent light and emit at a different wavelength. Such a system can help to screen out the effect of light generated in the bulk sample, especially if the emitted light is viewed through an appropriate filter. For example, blue light from luminol can be absorbed by a coumarin, which will emit yellow/green light. The fluorescer can be located on or near the signal sensing means. Alternatively, the fluorescer can be used as a marking agent dispersed in the bulk sample, so that only a localised generation of chemiluminescence is observable.

Although luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) is a particularly preferred chemiluminescent agent for use in the context of the invention, a range of alternative compounds and reactions

can be used. For example, other derivatives of 2,3-dihydro-1,4-phthalazinedione, such as the 6-amino derivative (isoluminol) can be used. Other examples are luciferin and acridinium esters. Such systems are generally described, for example, in GB 1552607, GB 2112779A and GB 2095830B. In general, chemiluminescent reactions tend to be short-lived, which may lead to time constraints on the assay procedure. It is therefore useful to enhance the effective duration of the light emission, e.g. by the addition of reagents that prolong or delay the emission. The enhancement of chemiluminescent reactions, using compounds such as substituted 6-hydroxybenzothiazoles, p-iodophenol, 4-phenylphenol and/or 2-chloro-4-phenylphenol and aromatic amines, is described in EP 87959, EP 116454 and GB 2162946A. The use of p-iodophenol is particularly preferred.

A further embodiment of the invention is a particle-based assay method which utilises:

(a) a first population of localisable particles (e.g. "magnetic" particles) bearing a specific binding partner for a chemical species under test;

(b) a non-particle-borne specific binding partner labelled such that the specific binding partner produces continuously within the test medium a precursor for a chemiluminescent reaction;

(c) a consumer reagent dispersed in the test medium which competes effectively for the precursor and thereby inhibits the production of chemiluminescence while the labelled specific binding partner remains dispersed in the test medium; and

(d) a second population of localisable particles bearing or containing a reagent which can react with the precursor to generate chemiluminescence,

and in which method co-localisation of the two particle populations adjacent a light sensitive film enables observation of chemiluminescence produced in an amount dependent on the extent to which the specific binding partner carried by the first population of particles has bound to the species under test.

In the foregoing embodiment, the label can be, for example, glucose oxidase reacting with glucose and oxygen in the test medium to produce hydrogen peroxide as the precursor, the consumer can be catalase enzyme, and the bound reagent can be horseradish peroxidase interacting with luminol in the text medium. If desired, the bound reagent can be carried on the surface or entrapped within the particles of the second population.

Sequential addition of the components in the test medium can be advantageous, e.g. to permit adequate time ("incubation") for the immunological binding reaction(s) to take place prior to the addition of a substrate required for the generation of a chemiluminescent signal. It will thus be appreciated that the continuous generation of light will only take place when the necessary substrates have been added. This addition will always be made before the solid phase is relocated to the detection zone or environment. The continuity of the light generation must last at least for the duration of the time taken to localise the solid phase and for the film to detect the light produced therefrom.

The incubation period needed for the specific binding reaction(s) to take place will dep nd on many factors,

such as the nature of the reagents and the analyte, their relative and absolute concentrations, and on physical factors such as temperature. These parameters are familiar to thos skilled in this art. In general, the incubation period will not exceed about an hour, and for most applications will lie in the range 30 minutes to one hour.

Localisation of the carrier material can be achieved in a wide variety of ways. As mentioned earlier, the sensitised solid phase can form part (or indeed, all) of the inner wall of the vessel in which the assay is completed. Alternatively, a single large portion of sensitised solid phase, such as a bead or peg, can be present in the vessel, and located adjacent the light-sensitive film at the recording stage. For example, plastics (e.g. polystyrene) beads ae commonly used as solid phases. Also, "plastic-coated" steel balls of diameter 2-5mm, i.e. having an applied coating of polymer such as polycarbonate which can be applied from solution, are used. These have the advantage that they can be positioned by the use of external magnets. In a preferred embodiment of the invention, however, the solid phase comprises a multitude of small particles.

It is possible to allow particles of solid phase to settle under gravity, although this necessitates a uniform suspension of the particles in the test medium being maintained, eg. by stirring, while the binding reaction is taking place. Centrifugation can be used as an alternative to gravitation. Alternatively, particles can be localised by passing a porous membrane or filter through the bulk of the test medium, thus sweeping the particles into a small volume. Another alternative is to use a particulate carrier mat rial which, under the influence of magnetism or another externally-applied

force, can be urged into a particular locality within the bulk of the test medium. The particles can be moved through a test medium by the ultrasonic generation of a drifting standing wave.

A particulate solid phase can be made of any suitable carrier material. So-called "magnetic" (i.e. particles that can be induced to move through a liquid under the influence of a magnetic field) particles of a variety of suitable sizes, ranging for example from less than about 1 to more than about 300μ, are available commercially. For gravity separation, particle sizes of at least about 50μ, and not greater than about 300μ, are preferred, depending on the density of the material. Commercially-available oxirane acrylic beads are very suitable, especially for assays involving labelled haptens. Techniques for sensitising and/or coupling such solid phase carriers to binding reagents, such as immunoglobulins, are well known standard techniques in the art, and form no part of the present invention.

The range of analytes to which the invention can be applied is vast. By way of example only, the following can be mentioned: Steroid hormones, such as those involved during pregnancy or which are influential on fertility, e.g. progesterone and oestrogens; urinary metabolites, such as oestrone-3-glucuronide and pregnanediol-3-glucuronide; peptide hormones, such as human chorionic gonadotrophin, luteinising hormone and follicle stimulating hormone; proteins, such as urinary albumin, beta-2 microglobulins and retinol binding protein; high and low-density lipoprotein, the detection of which is valuable in cardiovascular monitoring, and cardiac troponin; infectious disease markers, such as antigens of Chlamydia, Neisseria, Herpes, Candida and HIV1 (AIDS) and antibodies of Treponema (Syphilis), Rubella and

Toxoplasma, and immunoglobulins, such as monoclonal antibodies.

The test medium can thus be derived from many sources, especially body fluids such as urine, serum and plasma. The invention is especially applicable to the screening of the cell culture media for immunoglobulins, e.g. in the production of monoclonal antibodies.

The specific binding partners can be polyclonal or monoclonal antibodies or antigens, lectins, or hormones and their receptors. Such materials are well known in the art, many are available commercially, and their precise nature forms no part of the present invention.

If the sample suspected of containing the analyte is of variable nature, (e.g. serum and urine) or contains components which could interfere with the specific binding reactions or with the production of the observable signal, it can be advantageous to dilute the sample, e.g. with water or a buffer solution, before an assay method of the invention is applied, to reduce the likelihood of such interference.

Under some circumstances it is possible to enhance the sensitivity of an analytical procedure by removing at least the bulk of the test sample before observing the signal from the localised solid phase carrier. This is advantageous because many natural samples, such as blood serum, contain materials that can interfere with the signal generating reaction (so leading to a misleadingly low or negative observed result) or can initiate the signal generating reaction (so leading to a false positive observed result). This problem is already well known, and many conventional assays involve a series of "washing"

steps designed to separate completely the solid phase from the residual sample.

In an alternative embodiment of the present invention, the analytical method therefor includes the steps of:

a) localising the solid phase carrier material after an appropriate incubation period in the test sample;

b) removing at least the bulk of the liquid test sample;

c) adding a quantity of liquid medium containing such further reagents as are necessary to cause the light emitting reaction to occur; and

d) relocalising (if necessary) the particulate solid phase carrier material, and recording any light emitted from a point source.

A modification of the embodiment set forth in the immediately preceding paragraph includes the steps of:

a) localising the solid phase carrier material after an appropriate incubation period in a first liquid medium containing or comprising the test sample in the absence of the labelled reagent;

b) removing at least the bulk of the liquid test sample;

c) adding a quantity of a second liquid medium containing the labelled reagent;

d) relocalising the solid phase carrier material after an appropriate incubation period;

e) removing at least the bulk of the second liquid medium;

f) adding a quantity of liquid medium containing such further reagents as are necessary to cause the light emitting reaction to occur; and

g) relocalising (if necessary) the particulate solid phase carrier material, and recording any light emitted from a point source.

In a simplification of this last embodiment, the second and third liquid media can be one and the same, so that only a single liquid removal and replacement operation need be performed. Alternatively, the third liquid medium can be added to the vessel at the end of the second incubation step without removal of any of the second liquid medium.

As a further aspect of the invention, we have found, however, that it is unnecessary to wash the solid phase so thoroughly that all of the residual test sample and/or unbound labelled reagent is removed. Indeed, it is an advantage of the present invention that in this embodiment an analytical procedure can be provided which removes the bulk of the liquid medium using the minimum number of operations consistent with obtaining a reliable analytical result. The objective is to remove a substantial proportion of the liquid medium from the vessel. In general this proportion will be at least about 50%, and more usually at least about 90%, of the total volume of liquid in the vessel.

On option is to decant the supernatant liquid following localisation of the particulate solid phase carrier. The vessel in which the analytical reaction is

conducted is then replenished with another liquid medium. If necessary, this procedure can be repeated to provide an intermediate washing step (involving a relocalisation of the particulate carrier material prior to a second decantation and replenishment of the vessel) but, if possible, this should be avoided in order to minimise the number of operations required in the assay as a whole. In any event, the final replenishing medium must contain such reagents as are necessary to initiate or promote the chemiluminescent reaction.

As an alternate to decantation, the liquid medium can be removed from the vessel by pipetting or syphoning.

All of the separation techniques just described may lead to a problem of disposal of the liquid that has been removed from the vessel. In a most preferred embodiment of the invention, we prefer to use a wicking means to draw the bulk of the supernatant liquid from the reaction vessel by capillary action. The expression "wicking means" is used herein to convey any porous member that can be dipped into the vessel to absorb liquid therefrom and which member can subsequently to be removed from the vessel while leaving the localised particulate solid phase carrier substantially undisturbed and remaining in the vessel. Following removal of the wicking means containing the absorbed liquid, the vessel can be replenished with further liquid medium. If necessary, an intervening washing stage can be incorporated and a second wicking means used to remove the washing liquid following relocalisation of the particulate carrier material.

The wicking means can be made from any porous or fibrous material capable of absorbing liquid rapidly. The porosity of the material can be uni-directional (i.e. with pores running wholly or predominately parallel to the

longitudinal axis of the wicking means) or multi-directional (omni-directional, so that the wicking means has an amorphous sponge-like structure). Porous plastics material, such as polypropylene, polyethylene (preferably of very high molecular weight) polyvinylidene fluoride, ethylenevinylacetate, acrylonitrile and polytetrafluoro ethylene, can be used. Wicks can also be made from paper or other cellulosic materials such as nitro-cellulose. Materials that are now used in the nibs of so-called fibre-tipped pens are particularly suitable, and indeed we have found that nib units, which are currently available commercially for use in the manufacture of such pens, provide ideal wicking means for use in accordance with the invention. The wells of multi-well trays typically have a capacity of 300-400µl, and a nib unit having a diameter of about 5mm and a length of about 3cm can provide an ideal wicking means for rapidly removing a volume of up to, for example, about 400µl of a liquid medium from such a well.

The rate at which liquid is drawn into the wicking means from the vessel should be selected such that it is neither too fast nor too slow. Preferably the wicking means should be capable of absorbing the bulk of the liquid medium in the vessel within a matter of seconds, but should still have the capacity to absorb further liquid. In practice, if the wicking means absorbs liquid almost instantaneously and is thereafter immediately removed from the vessel, a significant film of liquid may be left adhering to the walls of the vessel. If the rate of absorbency is slightly slower, or if the wicking means is left in the vessel for a short period of time, any such residual liquid film can drain to the bottom of the vessel and be absorbed by the wicking means. Conversely, if the wicking means absorbs liquid only slowly (eg in minutes rather than seconds) this may unduly prolong the operation time, and preferably should be avoided.

As the wicking means is being used to absorb liquid medium from a vessel containing a particulate carrier material, it is preferable to localise the particulate carrier material in a vicinity out of contact with the wicking means in order to avoid the particulate carrier material adhering to, or indeed being drawn into, the wicking means by the liquid flow.

Conventional multi-well trays contain a plurality of wells arranged in an array. A corresponding array of wicking units in accordance with the invention can be used to remove liquid simultaneously from each member of the whole array of wells if desired.

A particular advantage of the invention is that liquid can be removed from the analytical vessel without risk of spillage or the need to dispose of the removed liquid in free-flowing form. The liquid will remain in the wicking means, and can be disposed of much more readily. An important advantage of a wicking means according to the invention is that when it has absorbed liquid medium it can be removed from the vessel and handled without risk of the liquid medium dripping. This is particularly important where analytical tests are being performed involving samples suspected of containing infectious disease organisms. The risk of aerosol formation is also minimised, which is an important aspect where liquid media containing infectious organisms are involved.

The dimensions and overall shape of the wicking means can be selected to remove the optimum volume of liquid from the analysis vessel while leaving the solid phase carrier (and hence any sensitive material bound thereto) as undisturbed as possible.

By way of example only, embodiments of the invention will now be described with reference to the accompanying drawings.

Recording Device

A "camera" recording device, useful in the context of the invention, is depicted in Figures 1 and 2 of the accompanying drawings. Figure 1 depicts a general view of the whole device, seen with the camera body lid in the open position. Figure 2 depicts an elevation in partial cross-section of the device as viewed on the line AA in Figure 1, with the lid of the camera body in the closed position.

The recording device shown in Figures 1 and 2 operates essentially on the principles as described in UK patent no. 2169704.

Referring to Figure 1, the recording device comprises a standard commercially available "Polaroid" instant camera "back" 100 including a hinged portion 101 openable to receive a film (not shown), and a slideable shutter 102 which can be pulled from the side 103 of the "back" to expose the film. Mounted on the top 104 of the camera "back" is a flat rectangular body 105 centrally perforated by a large rectangular opening 100 through which a film can be exposed to light when the shutter 102 is pulled open. The opening 106 accommodates a readily removeable plate 107 perforated by a plurality of vertical holes 108 arranged in a regular array and which can accommodate the wells of a standard commercially-available multi-well tray (not shown). Adjacent one end of the plate 107 is a small knob 108 attached to the upper surface 109 of the plate, enabling the plate to be grasped and lifted out of the body 105. The depth of the body 105 is greater than the

thickness of the plate 107 and the knob 108 does not extend above the top 110 formed by the body 105. A closeable lid 111 hinged at the rear 112 of the device can be swung downwards to close the top of the body 105. A light-tight seal is provided by a narrow continuous beading 113 which runs around the lower face 114 of the closeable lid 112 adjacent the edge 115 of the lid, and which beading engages in a corresponding continuous slot 118 which runs around the top 110 of the body 105.

Referring to Figure 2, the device is seen resting on a surface 200 by means of feet 201 attached to the bottom 202 of the camera "back" 100. The constructional details of the camera "back" are not shown, as these are entirely standard and not relevant to the invention. The lid 111 is shown in the closed position, with the beading 113 lying in the slot 116 at the top 110 of the body 105. The shutter 102 is shown in the closed position, and the perforated plate 107 is seen resting on the upper surface 203 of the shutter. When the shutter 102 is opened, the perforated plate 107 will drop and come to rest on a film (not shown) which will be lying just below the shutter. Figure 2 also shows a transparent multi-well tray 204 resting in the perforated plate 107 with the individual round-bottom wells 205 extending into the holes 108 in the perforated plate. The thickness of the plate is such that each well extends right to the bottom of each hole, and hence with the plate comes to rest on the film, after the shutter has been opened, each well will be in direct contact with the film. One of the wells is depicted as containing a liquid sample 206 having a particulate solid phase 207 localised at the base of the well. The quantity of solid phase 207 is grossly exagerated for the purposes of the drawing, whereas in practice the volume accupied by the particulate solid phase when localised will be very small.

At the end of an analytical procedure conducted in a multi-well tray resting in the perforated plate, a particulate solid phase can be localised at the bottom of each well (e.g. by magnetic influence) and the perforated plate placed inside the recess in the camera body and the lid closed. The shutter can then be opened to expose the film and allow chemiluminescent light produced in a chemical reaction induced by a labelled component attached to the localised solid phase to be recorded on the film.

By way of example only, various ways of producing point sources in accordance with the invention, are illustrated in Figures 3 to 6 of the accompanying drawings.

Referring to Figure 3, a single transparent well 300 (forming part of an array of such wells in a multi-well plate) is seen in cross-sectional elevation sitting in its opaque mounting plate 301. The diameter of the well decreases sharply towards the bottom end 302 and defines a narrow conical region at the base of the well. The mounting plate 301 is seen sitting directly on top of a light-sensitive film 303 and the well 300 extends right to the bottom 304 of the mounting plate so that the tip 302 of the well is also directly in connect with the film. The vertical sides 305 of the hole 306 in the mounting plate 301 which accommodates the well 300 define an exposed region 307 of the film, having a diameter substantially greater than that of the well at its bottom end. The well contains a volume 308 of liquid reagent and a small quantity 309 of a finely divided particulate solid phase which has been localised, e.g. by magnetism, in the bottom of the well. Due to the small quantity of particulate solid phase present and the internal dimensions of the well, the particulate solid phase is concentrated in a very small volume at the bottom of the

well, and if the solid phase carries a reagent capable of emitting light as a result of a chemiluminescent reaction, the light is all evolved in a region which is much smaller than the area of exposed film and therefore acts as a point source. Even though the accumulated solid phase is only separated from the film by the thickness of the transparent material forming the well wall, this is nevertheless sufficient to allow the light spot formed on the film to be of relatively greater of lesser diameter depending on the amount of light generated from the solid phase.

The use of an aperture to establish a point source of light is depicted in Figures 4, 5 and 6. Referring to Figure 4, a single tapering, round-bottomed, transparent well 400 is seen in cross-sectional elevation in its opaque mounting plate 401. Immediately beneath the base 402 of the plate 401 and in contact with the bottom 403 of the well, is a thin perforated opaque plate 404 having a small perforation 405 immediately beneath the tip of the well. The width of the perforation 405 is substantially less than the diameter of the hole 406 in the mounting plate which accommodates the well. Below the perforated plate 404 is a light-sensitive film 407 separated therefrom by a small distance X. The well contains a volume 408 of liquid reagent, and a single large opaque bead 409 as solid phase which is seen resting in the bottom of the well and substantially filling the lower portion of the well. The diameter of the bead 409 is substantially greater than the size of the aperture 405. If the surface of the bead carries bound reagent that can be emit light as a result of a chemiluminescent reaction, light from the surface of the bead immediately adjacent the aperture can pass therethrough and onto the film. The diameter of the spot produced on the film will vary

depending on the amount of light emerging through the aperture, and the magnitude of distance X.

An alternative embodiment is seen in Figure 5, where the well 500 is flat-bottomed and the inner surface 501 of the well in the lower regions is sensitised with reagents and acts as a permanently localised solid phase. If chemiluminescent light is emitted from labelled reagents that have become bound to this sensitised surface, the whole lower region will emit light but only the light passing through the aperture 502 will fall onto the film 503 below. As before, the diameter of the spot produced on the film will be dependent on the amount of light passing through the aperture.

Another embodiment is seen in Figure 6, where the well 600 is round-bottomed but relatively wide at its base, and contains a substantial mass 601 of particulate solid phase covering almost the whole of the bottom 602 of the well. An aperture 603 defines a much smaller region through which light can pass, and which therefore acts as a point source.

Referring to Figure 7, a pair of wells 700 and 701 in an array in a transparent multi-well plate, are seen in cross-sectional elevation sitting in an opaque mounting plate 702. The general arrangement is as described above with reference to Figure 4. In this instance, however, the opaque perforated plate 703 which provides the aperture 704 beneath each well is separated from the film 705 below by another thicker opaque perforated plate 706 in which the holes 707 have a substantially larger diameter than the apertures 704 and, indeed, as depicted in th drawing having the same diameter as the holes 707 in the mounting plate 702 which accommodate the wells. Each hole in the separating plate 706 defines a region 708

of the film which can be exposed to any light passing through the aperture. Because the separating plate is opaque, each defined region of the film is separated from the regions associated with surrounding wells and the risk of light from one aperture impinging on the area of film beneath another adjacent aperture is minimised.

Referring to Figure 8, the composite elements for a holder for a multi-well tray are depicted in "exploded" form. For simplicity in the drawing, the transparent tray 800 is shown as having an array of 12 wells in a 4 x 3 arrangement, but in practice, the number of wells would normally be substantially greater. The multi-well tray can be accommodated in an opaque perforated mounting plate 801 having a hole 802 to accommodate each well. The thickness 803 of the mounting plate is identical to the external depth of the wells. Beneath the mounting plate 801 is an opaque apertured plate 804 having a corresponding number of small apertures 805 each lying on the vertical axis of a hole in the mounting plate 801. Beneath the aperture plate 804 is an opaque spacing plate 805 also having the same number of holes which in this instance are of same diameter as the holes in the mounting plate 801. The thickness 807 of the spacing plate 806 defines the distance separating each aperture 805 from a light-sensitive film (not shown) that may be placed directly beneath the spacing plate.

Example 1

A "camera" recording apparatus basically as just described was used in the following hCG assay.

MATERIALS

a) Solid Phase

Commercially-available macroporous polystyrene particles (Dyno: Dynospheres XP-6006), average size 2.6µm, within which is an even distribution of iron oxide. These uniform, spherical beads have a hydroxyethylmethacrylate based outer shell derivitised with pendant tosyl groups. The tosyl moieties can readily undergo nucleophilic substitution by amino groups such as are present on the outer surface of a protein, leading to immobilisation. These were sensitised with a mouse anti-hCG monoclonal antibody, by the following procedure.

To an aliquot of a suspension containing Dynospheres XP-6006 (10mgs) was added boric acid buffer (0.005M, pH 9.5, 0.5ml). To this was then added the antibody solution (200µg in 1ml PBS, pH 7.2) followed by boric acid buffer (0.5M, adjusted to pH 9.5 with NaOH, 0.5ml).

This was left overnight on an orbital shaker at room temperature to couple. The beads were then sedimented with a magnet and the protein solution aspirated. They were washed once with PBS (5ml), and the coupling procedure is repeated, but this time using BSA (20mgs) to couple to any unreacted tosyl groups. The beads were then washed overnight in phosphate buffer (0.25M KH_2PO_4, adjusted to pH8 with NaOH containing 0.5% BSA and 0.1% Tween 20, 5ml) to remove any adsorbed antibody from the bead surface. Finally, the beads were washed in the same buffer (2 x 5ml) and then washed in PBS (2 x 5ml) and stored in a known volume of PBST buffer containing 0.1% BSA + 0.01% Thimerosal preservative at 4°C.

b) Conjugate

The same mouse anti-HCG monoclonal antibody was conjugated with horseradish peroxidase enzyme, using a conventional procedure, in PBST buffer.

c) Analyte

A range of urine samples containing known concentrations of hCG ranging from zero up to 405 mIU/ml.

d) Luminol Cocktail

A solution in 0.1M Tris/HCl pH 8.5 buffer, of 1mM luminol, 1mM para-iodophenol and 10mM urea peroxide.

e) Camera Film

Polaroid Type 612 high-contrast black-and-white 20,000 ASA film.

PROCEDURE

To each of a series of round-bottom wells in a conventional transparent plastics-moulded multi-well ("Micro- titre") tray was pipetted:

50µl of analyte sample from the range of concentrations stated above;

50µl of conjugate; and

50µl of the solid phase (0.05% solid, giving 25µg of solid phase per well);

and the position of each sample in the array of wells noted on the tray.

The tray was covered with plastic film and shaken on a laboratory shaker at room temperature for 1 hour. The tray was then placed on top of an array of small magnets, corresponding to the array of wells in the tray, in order to localise the particulate solid phase at the bottom of each well. After about 2 minutes, the plastic film was removed and (with the array of magnets held in place beneath the tray) the supernatant liquid was decanted from each well and the tray blotted, and the tray separated from the magnets. 200µl of PBST buffer was added to each well, and the tray left for 1 hour without shaking. The solid phase was then relocalised at the bottom of each well and the decanting and washing step repeated. Thereafter, the tray was removed from the array of magnets and 100µl of the luminol cocktail added to each well. After 1 minute, the tray was replaced on the array of magnets for 2 minutes and then gently removed and placed in the perforated plate of the camera, and the complete unit placed in the camera and the lid closed. The shutter was opened and the film exposed for 5 minutes.

Figures 9 and 10 of the accompanying drawings illustrate the typical result obtained. A typical photograph is shown in Figure 9, with two parallel arrays of wells having been used in the experiment to provide duplicate results. The increasing diameter of the spots on the exposed film correlate with the increasing concentration of analyte in the samples. The graph in Figure 10, in which the spot diameter (X) has been plotted against the hCG concentration (Y) expressed in MIU/ml, shows that within experimental error there is a reasonably linear correlation between the log concentration and the spot diameter.

Example 2

This example illustrates the screening of supernatants from monoclonal antibody cell line cultures, and the partial removal of sample between two incubation steps.

MATERIALS & METHODS

Solid Phase

Dynosphere XP-6006 beads as described in Example 1 were used as the particulate solid phase. These were sensitised with Dakopatts Z259 rabbit anti-mouse immunoglobulins.

Rabbit Anti-Mouse/Biotin

The Dakopatts Z259 (rabbit anti-mouse immunoglobulins) reagent was biotin labelled through reaction with biotin N-hydroxy succinimide (BNHS), at pH 7.5. Un-reacted BNHS was removed by extensive dialysis.

Avidin HRP Conjugate

Commercial Sigma No. A3151 horseradish peroxidase labelled Avidin conjugate was used as recommended by the manufacturers.

Wicks

Two types of "wick" were used to remove sample fluid during the two-part incubation. These were commercially-available fibre-tip pen nib units:

a) Omnidirectional porous polyethylene nib units of cylindrical shape having a regular conical pointed tip, manufactured by Porex Technologies, Fairburn, Georgia, USA. These were capable of absorbing 200µl of water in about 14 seconds.

b) Predominately undirectional, fibrous "chisel-shaped" nib units, manufactured by Stylex SA, Lamone-Lugano, Switzerland. There were capable of absorbing 200µl of water in less than 1 second.

HS Medium

"HS medium" is 5% horse serum in RPMI 1640 which consists of

1) 500ml of commercial RPMI 1640 medium (Flow laboratories)
2) 25mls horse serum
3) 5mls Penicillin streptomycin (Penicillin 5000 IU/ml and streptomycin 5000µg/ml)
4) 5mls Sodium Pyruvate, 100mM
5) 5mls L-Glutamine, 200mM

Assay Procedure

1. 5µl of the 0.625% Dynosphere suspension was incubated with 15µl of the antibody sample diluted in 5% HS medium in the round-bottom wells of a conventional transparent plastics-moulded "Microtitre" tray for 30 minutes at room temperature on a shaker. The sample was a mouse monoclonal antibody solution, containing a range of concentrations from 1ng/ml up to 500µg/ml, in HS medium, or bland HS medium as a control.

2. After the incubation the Dynospheres were localised with a magnet, and the excess antibody solution wicked away using either a Stylex or Porex wick.

3. 30µl of a preformed avidin HRP-biotin (RaM) complex was then added to each well, resuspending the Dynospheres. (The complex consisted of equal volumes of 1/500 rabbit anti-mouse/biotin and 1/2000 Avidin HRP conjugate mixed together and left for a minimum of 30 minutes). The Dynospheres were incubated with the complex for 30 minutes at room temperature on a shaker.

4. 150µl of luminescent reagents was then added and the Dynospheres were bottom-localised on a magnet for 2 minutes. The reagents consisted of:

10mM Luminol	)	Volume ratio
5mM P-iodophenol	)	= 1:1:1
100mM Urea Peroxide	)	

and 15µl of commercial black ink was added per 1ml of mixture before dispensing into the wells.

5. After magnetic localisation of the Dynospheres, the wells were exposed to 20,000 ASA Polaroid print film in a camera as described above in Example 1, for about 1-2 minutes. The film was allow to develop for 1 minute, at which time each positive result was revealed as a white spot on a black print.

RESULTS

The assay could detect antibodies at levels from 1ng/ml through to 500µg/ml, whilst the blank HS medium gave no spot. Within certain limits, the spot size was

directly related to concentration of the analyte, but beyond this limit there was a plateau effect, and then spot size tended to decrease at very high analyte concentration. A typical print is shown in Figure 11 of the accompanying drawings, and the results from a typical test depicted graphically in Figure 12.

EXAMPLE 3

The "camera" recording apparatus of Examples 1 and 2 was used, with slight modification, in an assay for mouse immunoglobulin G (IgG). A cut-down, flexible transparent "Microtitre" multi-well plate was carried in a removable plate which had been fitted with an aperture plate and spacer plate as shown in Figures 4, 7 and 8 of the accompanying drawings. With this arrangement, each well was placed over an aperture of 2mm diameter spaced either 3mm or 1.8mm away from the surface of the film, depending on the spacer plate fitted. As this holder was removable, it was interchangeable with the standard holder used in Examples 1 and 2. The holes in the spacer plate were circular and had a diameter of 8mm, thus defining a nominal maximum spot size of 8mm diameter.

MATERIALS

a) Solid Phase

Plastic coated steel ball bearing (3mm dia), hereinafter termed "beads", were sensitised with Dakopatts Z259 rabbit anti-mouse immunoglobulin by the following procedure.

The immunoglobulin was diluted to 47 µg/ml in 0.05M carbonate/bicarbonate buffer, pH 9.6, and this solution was added to the beads at the rate of 50µl per bead (e.g.

5ml for 100 beads). This sensitising solution was left in contact with the beads for 16 hours (overnight) at room temperature, after which it was decanted off and beads were rinsed twice in distilled water.

Conjugate

A commercially available conjugate consisting of peroxidase-conjugated rabbit immunoglobulin to mouse immunoglobulin (Dako, code P161) was used. Dilutions were made with PBST containing 0.5% BSA.

Analyte

A range of samples were prepared by dilution of a stock solution of purified monoclonal mouse IgG in PBST containing 0.1% BSA to give concentrations of:-

100 ng/ml
10 ng/ml
1 ng/ml
0.1 ng/ml
0.01 ng/ml

Luminol Cocktail

The luminescent reagent mixture consisted of a solution of 1mM luminol, 1mM para-iodophenol and 10mM sodium perborate.

Camera Film

The "camera" recording apparatus was loaded with Polaroid Type 612 high-contract black-and-white film, 20,000 ASA.

PROCEDURE

Wells of a cut-down tray were dosed with 150µl volumes of the sample solutions, 3 for each concentration. An additional set of 3 wells were dosed with PBST/0.5% BSA diluent, as a negative control. A sensitised bead was added to each well.

The tray (plus samples and beads) was placed on a shaker in an incubator at 37°C and incubated for 30 minutes, after which the samples were aspirated-off. To do this, the tray was placed on a block bearing an array of small magnets positioned such that each bead was held at the side of its well, displaced sufficiently to allow a narrow tube to be inserted to the bottom of the well. Each well was filled with PBST, which was then aspirated-off in the same way, to give a minimal rinse.

Next, 150µl volumes of conjugate (diluted $^{1}/_{100}$) were added to each well and bead, and again the tray was incubated with shaking for 30 mins at 37°C. After this incubation step, conjugate was aspirated off as before, and each bead was rinsed seven times with PBST.

Each rinsed bead was then covered with 150µl of luminol cocktail, and the tray was placed in a "camera" apparatus with a standard tray carrier. The film was exposed to the wells (containing beads plus cocktail) for 30 seconds. The tray was transferred to a modified carrier with 2mm apertures and 3mm space plate, replaced in the camera and exposed to film for 2 minutes. This was repeated with another space plate of 1.8mm and a fourth exposure was made with the last arrangement, but for 1 minute. Then various r cording conditions are summarised in Table 1.

TABLE 1

CONDITIONS			
PRINT NO.	APERTURE RESTRICTION	SPACE ABOVE FILM	EXPOSURE TIME
1	None	None	30 secs
2	2mm	3mm	120 secs
3	2mm	1.8mm	120 secs
4	2mm	1.8mm	60 secs

RESULTS

Typical prints are seen in Figure 13 of the accompanying drawings.

The-30 second exposure (print A) with no aperture restriction showed that the 100 ng/ml samples produced most light, whilst at the other extreme the zero controls produced a barely detectable level of light. In the absence of an aperture restriction, however, the high light yields gave a blurred, overlapping image. At 1 ng/ml there was a sharp, full spot, but below that value the spots were ragged and ill-defined.

Print B gave a clearer picture. The 1 ng/ml, 10 ng/ml and 100 ng/ml samples gave identical full spots, whilst the 0.1 ng/ml samples gave faint blurred spots, and the 0.01 ng/ml samples gave a barely detectable blur.

When the space between aperture and film was reduced to 1.8mm (Print C), a greater resolution was obtained, to the extent that the 1 ng/ml samples gave a reduced spot size, the 0.1 ng/ml samples gave a still smaller size, and the 0.01 ng/ml samples were detectabl with very small spots.

The zero control gave barely-perceptible faint background spots of lower intensity than the 0.01 ng/ml sample.

By reducing the exposure time from 120 seconds to 60 seconds (print D), the sensitivity and range were changed slightly, with the 100 ng/ml samples giving the only full-sized spots, and the spots for all of the others distinctly reduced in diameter in progression.

Claims

1. An assay method for determining the presence of an analyte in a sample by means of specific binding, using a solid phase carrier material on which is immobilised a first binding reagent having specificity for the analyte, and a labelled reagent which participates in either a "sandwich" or a "competition" reaction with the first reagent in the presence of the analyte, and the label participates in a chemiluminescent reaction the light output from which is used as a means for determining the extent (if any) to which the labelled reagent has become bound to the solid phase, the light is recorded on a light-sensitive film and the light is derived from a point source in such a manner that the size of the image formed on the film provides a measure of the quantity of the analyte in the sample.

2. An assay method according to claim 1, wherein the area of solid phase presented to the light-sensitive film is not greater than about 50% of the area of film exposed to the light source.

3. An assay method according to claim 2, wherein the area of solid phase presented to the light-sensitive film is not greater than about 25% of the area of film exposed to the light source.

4. An assay method according to any one of the preceding claims, wherein the solid phase itself defines the point source.

5. An assay method according to claim 4, wherein the solid phase is particulate and the point source is

established by localising the particulate solid phase in a small region in a vessel in which the assay is conducted.

6. An assay method according to claim 5, wherein the creation of the point source is achieved by localising a particulate solid phase at the bottom of a well having a substantially narrower cross-section at the base then at the top.

7. An assay method according to any one of claims 4 to 6, conducted in one or more wells of a conventional multi-well tray, and wherein the light-sensitive film is placed in direct contact with the bottom of the wells in the tray to record the luminescent signal.

8. An assay method according to any one of claims 1 to 3, wherein the point source is defined by an aperture placed immediately adjacent to the exterior of the vessel in which the assay is conducted, and the light-sensitive film is spaced away from the aperture.

9. An assay method according to claim 8, wherein a perforated spacer is provided between the aperture and the film in order to maintain accurately a distance between the aperture and the film.

10. An assay method according to claim 8 or claim 9, conducted in one or more wells of a conventional multi-well tray, and wherein the aperture is provided by a perforated plate which can be situated directly beneath the tray such that there is an aperture directly beneath each well.

11. An assay method according to any one of claims 8 to 10, wherein the aperture, or each aperture, has a maximum dimension of at least about 0.5mm.

12. An assay method according to any one of claims 8 to 11, wherein the aperture, or each aperture, has a maximum dimension of not greater than about 3mm.

13. An assay method according to any one of claims 8 to 12, wherein the aperture, or each aperture, has a maximum dimension of about 1 to about 2mm, and the distance between the aperture and the light-sensitive film is at least about 0.5mm but not greater than about 5mm.

14. An assay method according to claim 13, wherein the distance between the aperture and the film is in the range to 1 to 3mm.

15. An assay method according to any one of the preceding claims, wherein after appropriate incubation with a sample and a soluble or dispersible labelled specific binding reagent, a particulate solid phase carrier material bearing an immobilised specific binding agent is localised within a liquid medium adjacent to a light-sensitive film to enable light to be detected from a point source, the label being capable of participating in a chemical reaction that results in the generation of chemiluminescent light and the liquid medium containing such additional reagents as may be necessary to cause the reaction to occur.

16. An assay method according to any one of claims 1 to 14, including the steps of:

(a) preparing an aqueous assay medium containing;

i) a particulate solid phase carrier material on which is immobilised a specific binding reagent for the analyte to be determined, the particulate solid phase carrier being

suspendable in the aqueous assay medium but readily localisable therein;

ii) a sample suspected of containing the analyte; and

iii) a labelled reagent soluble or dispersible in the assay medium which is either a second specific binding reagent capable of participating with the analyte and the first specific binding agent in a "sandwich" reaction, or is the analyte itself or an analogue of the analyte, and the label is a chemiluminescent reactant;

(b) incubating the assay medium to enable the labelled reagent to become bound to the particulate solid phase carrier;

(c) localising the particulate solid phase carrier within the assay medium;

(d) replacing or diluting the assay medium using a liquid medium, containing or together with such additional reactants as may be necessary to cause the chemiluminescent reaction to occur; and

(e) measuring on a light-sensitive film the chemiluminescent light emitted from a point source comprising label bound to the particulate solid phase carrier in the localised state.

17. An assay method according to claim 16, modified by performing the incubation in two stages, involving an initial incubation of the particulate solid phase carrier material and the sample in the absence of the labelled reagent, at least the bulk of the sample then being

removed and replaced by a liquid medium containing the soluble or dispersible labelled reagent, and a second incubation being performed, whereafter steps (c) to (e) of the above sequence are conducted.

18. An assay method according to any one of claims 1 to 14, utilising a specific binding partner which is labelled such that the label coninuously generates a chemiluminescent signal in the test medium, which signal is masked or quenched or otherwise suppressed while the labelled binding partner is uniformly dispersed within the test medium, and in which method, following the binding reaction, a local concentration of the particulate carrier material is established in the test medium such that the local concentraction of complexed labelled binding partner carrier by the particles provides a local light flux that is sufficient to overcome the local signal-suppressing ability of the test medium and hence to provide a detectable chemiluminescent signal the magnitude of which is dependent on the extent of binding that has taken place between the immunogenic species under test and the specific binding partner carrier by the particles.

19. An assay method according to any one of the preceding claims, wherein the chemiluminescent agent is luminol or a similar derivative of 2,3-dihydro-1,4-phthalazinedione.

20. An assay method according to claim 19, wherein the chemiluminescent reaction is enhanced by the presence of para-iodophenol.

21. An immunoassay method according to any one of the preceding claims.

22. An assay method according to any one of the preceding claims, conducted in one or more wells of a conventional transparent multi-well micro-analytical tray.

23. An assay method according to any one of the preceding claims, wherein the solid phase is particulate and removal of liquid from a vessel in which the assay is conducted is effected by wicking.

24. A device for determining the result of a specific binding assay, utilising the light output of a chemiluminescent reaction as a means for determining quantitatively the presence of an analyte in a sample, comprising a holder for a multiplicity of reaction vessels, the holder comprising a first plate having an array of holes therein for receiving an array of reaction vessels, a housing for receiving the holder, the housing being adapted to prevent entry of stray light and including an openable cover, means for holding a photographic film adjacent the underside of the plate and a removable shutter for interposition between the film and the plate, said removable shutter being moveable between a closed position and an open position in which latter position the film is exposed in use, and interposed between the base of the holder and shutter is a perforated second plate the individual perforations of which each provide an aperture beneath an individual hole in the holder, each aperture having an area substantially less than that of its associated hole in the holder, together with means for maintaining a predetermined distance between the film and the perforated plate when the shutter is moved to its open position.

25. A device according to claim 24, wherein the distance maintaining means comprises a perforated third plate in which the perforations are substantially larger than the

apertures in the second plate, each perforation in the third plate being situated directly beneath an aperture in the second plate.

26. A device according to claim 25, wherein the triple combination of the plates is arranged (a) to rest on and be supported by the shutter when the latter is in its closed position and (b) to drop on to the photographic film when the shutter is moved to its opened position.

27. A device according to any one of claims 24 to 26, wherein each aperture has a maximum dimension of at least about 0.5mm.

28. A device according to any one of claims 24 to 27, wherein each aperture has a maximum dimension of not greater than about 3mm.

29. A device according to claim 27 or claim 28, wherein each aperture has a maximum dimension of about 1 to about 2mm, and the distance maintained between the aperture and the light-sensitive film is at least about 0.5mm but not greater than about 5mm.

30. A device according to claim 29, wherein the distance maintained between the aperture and the film is in the range to 1 to 3mm.

31. An assay method according to claim 1, substantially as hereinbefore described with reference to any one of Examples 1 to 3.

32. A device according to claim 24, substantially as hereinbefore described with referenc to Figures 1, 2, 7 and 8 of the accompanying drawings.

Published 1988 at The Patent Office, State House, 66/71 High Holborn, London WC1R 4TP. Further copies may be obtained from The Patent Office,